Human Brain Surrogates: Models or Distortions?

By Monika Piotrowska

Although neurological disease and mental illness can cause terrible human suffering, strategies for researching their causes and cures is not obvious. Invasive brain research on actual human beings is clearly not an option for ethical reasons. As a result, neuroscientists have been inspired to model living human brains outside of living human beings. Hank Greely refers to such research subjects as "human brain surrogates" and divides them into four categories: 1) genetically edited non-human animals, 2) human/non-human brain chimeras, 3) human neural organoids, and 4) living *ex vivo* human brain tissues. In his target article, Greely argues that the creation of human brain surrogates is pushing us towards the following problem:

> When we avoid unethical research by making living models of human brains, we may make our models so good that they themselves deserve some of the kinds of ethical and legal respect that have hindered brain research in human beings. If it looks like a human brain and acts like a human brain, at what point do we have to treat it like a human brain—or a human being? (Greely 2020)

This is an important question that may one day require serious deliberation, but that day isn't on the visible horizon. At least, it's not on the horizon with respect to categories one and two

from above, *viz.*, 1) genetically edited non-human animals and 2) human/non-human brain chimeras. Largely, this is due to difficulties in assessing "how whole, living, integrated brains function inside living non-human animals when changes have been made that might tell us something about humans" (Greely 2020). I want to offer two arguments to explain this difficulty, which will also serve to explain why Greely's worry is misplaced. First, I'll argue that the nonhuman bodies of host models produce distortions in representing the functional mechanisms used to produce human behavior. And second, I'll argue that changing host model phenotypes results in behavioral changes that are difficult to interpret.

Turning to the first point, consider an experiment in which mice were genetically engineered to carry a FOXP2 variant that causes language disorders in humans.[1] Known as "the human language gene," FOXP2 fell under the spotlight when it was identified as the cause of a severe speech and language disorder affecting about half the members (through four generations) of a large family in London. Researchers soon learned that the gene was highly conserved evolutionarily, which is to say that there are only three amino acid differences between the gene as found in humans and as found in mice. Despite that similarity, however, when the human variant was transferred to mice, the "humanized" mice did not exhibit apparent vocalization deficits. The disappointing outcome led to skepticism, with some researchers concluding that "[r]ecapitulation of specific human gene mutations does not . . . predictably recapitulate gene function in animal models" (Zhao 2018, 832). In other words, the nonhuman body of the surrogate mouse—its genetics, development, and overall physiology—

[1] Much of this and the next two paragraphs is from earlier work, see Piotrowska 2013 for specific citations.

distorted the anticipated effects. The functional mechanisms used to produce human behavior were not replicated in the model even though they were thought to be the same mechanism.

While genetically editing non-human animals is one way of making human brain surrogates, an alternative is to transfer entire human cells into the surrogate model. But making human/non-human brain chimeras in this manner is also riddled with obstacles. For example, human embryonic stem (ES) cells often fail to functionally integrate into the host when they are transferred into preimplantation rodent embryos. Furthermore, it's not uncommon for inserted human cells to disappear altogether post transfer. Differences in the rate of development between the two species is the likely culprit. Human ES cells grow at a slower pace than mouse ES cells and are likely to get outcompeted inside a mouse host. For this reason, it's unlikely that making human/non-human brain chimera models is going to show us much, since the attempt to create a model is effectively ruined when the inserted human cells are outcompeted by the host's.

But even if we could get the human genes or cells to functionally integrate into the host, the physiology of the host's body can be an obstacle to modeling human behavior. It's awfully difficult to model human language disorders in rodents that don't generate sound by oscillation of the vocal folds but, rather, by an aerodynamic whistle. Similarly, rodents lack the motor control required to coordinate muscle movements in the lungs, tongue, and lips, which are all necessary for articulation of human speech. In short, attempting to model human behaviors produced from the workings of the brain using animals with dramatically different physiological mechanisms leaves us with a model we simply don't understand. For these reasons, I don't believe the worry that surrogate models may soon be "so good that they themselves deserve

some of the kinds of ethical and legal respect that have hindered brain research in human beings" (Greely 2020) expresses a real worry. The current state of research, at least with regards to the first two categories of human brain surrogacy, is simply too primitive.

Turning, then, to my second point, which is that rodent behavior is difficult to meaningfully interpret. This may sound like an easy problem to resolve, but it's more difficult that it first appears. Consider, for example, the use of rodents for understanding the biological significance of adult-generated neurons in the hippocampus. Using knock-out mice, researchers have increased (at other times decreased) adult hippocampal neurogenesis and then tested for behavioral changes in a laboratory setting. Both mice that experienced *90% loss* of new hippocampal neurons (Jabolkowski et al. 2009) and ones that had a *two-fold increase* in adult-generated neurons (Sahay et al., 2011bb) were behaviorally indistinguishable from controls on hippocampal-dependent tasks such as object recognition, spatial learning, and fear conditioning. Such surprising results might lead one to conclude that there is no causal connection between adult hippocampal neurogenesis and behavior. However, when another group of researchers tested the deficient mice—the ones with a 90% loss of new hippocampal neurons—on species-typical behaviors that do not require training, e.g., nest-building behavior, digging, and object burial, they observed a specific deficit (Jedynak et al. 2012). This might be due to the fact that the artificial nature of the laboratory setting inevitably limits the types of standard behavioral tests available. As neuroscientist Fernando Nottebohm has said, when you "[p]ut rats and mice into little plastic boxes . . . you will never fully comprehend why they do what they do" (Oppenheim 2019, 272). But the odd outcomes may be the result of something else. We simply don't know.

Consider another example: The Morris Water Maze, which is considered the gold-standard for memory testing in rodents. Even with this gold standard:

> [F]actor analysis of behavior data from 1400 mice revealed that only about 13% of the variance in this test is explained by memory, while most of it is explained by behaviors such as wall hugging (thigmotaxis) and passive floating, which are thought to represent emotional and motivation aspects. (Kafkafi et al. 2018, 227)

The failure of the water maze to test for memory might be explained by the fact that the house mouse is not well adapted to wet environments and swimming, but it could be something else. Again, we simply don't know enough about how mice act outside of the laboratory setting.

The task of interpreting rodent behavior is further complicated by the fact that the world from a human point of view is considerably different from that of a rodent: olfactory cues are very important for rodents; they are sensitive to near ultraviolet light; highly sensitive to movement and changes in light intensity; and they communicate in the ultrasound range. As Neri Kafkafi et al. (2018) argue, "such differences may hinder experimenters from detecting subtle environmental effects impacting on behavior" (223). The point is that we simply don't understand enough about rodent behavior to understand what they are doing most of the time, and that problem is made worse when we start manipulating rodent phenotypes in an effort to see how new behavior compares with old. If we want to understand, and hopefully, cure, diseases of the human brain, we should first validate and refine rodent behavioral constructs.

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